AF

CERTIFICATE OF MAILING BY FIRST CLASS MAIL (37 CFR 1.8) Applicant(s): L. Mansfield, M. Rossano, A. Murphy and R. Vrable			Docket No. MSU 4.1-458	
Application No. 09/513,086	Filing Date 02/24/2000	Examiner Joseph T. Woitach	Customer No. 21036	Group Art Unit 1632

Invention: **VACCINE TO CONTROL EQUINE PROTOZOAL MYELOENCEPHALITIS IN HORSES**

I hereby certify that this **REPLY BRIEF UNDER 37 CFR 41.41**
(Identify type of correspondence)

is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to "Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450" [37 CFR 1.8(a)] on **November 16, 2006**
(Date)

Tammi L. Taylor
(Typed or Printed Name of Person Mailing Correspondence)

Tammi L. Taylor
(Signature of Person Mailing Correspondence)

Note: Each paper must have its own certificate of mailing.

If any additional fees are required, please charge Deposit Account No. 13-0610.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : 09/513,086 Confirmation No. 4724

Applicants : Linda S. Mansfield, Mary G. Rossano, Alice J. Murphy, and Ruth A. Vrable

Filed : February 24, 2000

Title: VACCINE TO CONTROL EQUINE PROTOZOAL MYELOENCEPHALITIS IN HORSES

TC/A.U. : 1632

Examiner : Woitach, Joseph T.

Docket No. : MSU 4.1-458

Customer No. : 21036

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REPLY BRIEF UNDER 37 C.F.R. § 41.41

Sir:

This is a Reply Brief in response to the Examiner's Answer mailed October 17, 2006 in the above entitled application. The claims on appeal are set forth as in the Claims Appendix.

(1) Real Party in Interest

The real party in interest is the Board of Trustees operating Michigan State University, East Lansing, Michigan, a constitutional corporation of the State of Michigan, which is the assignee of the above entitled application.

(2) Related Appeals and Interferences

Related Appeals are described in the Supplemental Appeal Brief, filed June 27, 2006.

(3) Status of Claims

Claims 4, 13, 46 and 50 are pending in the application. Claims 1-3, 5-12, 14-45, 47-49 were cancelled. Claims 4, 13, 46 and 50 were rejected. No claims have been allowed. Claims 4, 13, 46 and 50 are on appeal.

(4) Status of Amendments

No amendments have been filed subsequent to final rejection.

(5) Summary of Claimed Subject Matter

The claimed subject matter is summarized in the Supplemental Appeal Brief, filed June 27, 2006.

(6) Grounds of Rejection to Be Reviewed on Appeal

(A) The Examiner rejected Claims 4, 13 and 46 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

(B) The Examiner rejected Claims 4, 13 and 46 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention, since a disclosure cannot teach one to make or use something that has not been described.

(C) Claims 4, 13, 45, 46 and 50 were rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

(7) Argument

(A) The Examiner rejected Claims 4, 13 and 46 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

What is conventional or well known to one of ordinary skill in the art need not be disclosed in detail. *See Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d at 1384, 231 USPQ at 94. >See also Capon v. Eshhar, 418 F.3d 1349, 1357, 76 USPQ2d 1078, 1085 (Fed. Cir. 2005) ("The 'written description' requirement must be applied in the context of the particular invention and the state of the knowledge…. As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution."). If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the

adequate description requirement is met. *See, e.g., Vas-Cath*, 935 F.2d at 1563, 19 USPQ2d at 1116; *Martin v. Johnson*, 454 F.2d 746, 751, 172 USPQ 391, 395 (CCPA 1972) (stating "the description need not be in *ipsis verbis* [i.e., "in the same words"] to be sufficient").

While there are the examples in the specification directed to the naturally occurring proteins are prophetic, as explained by the Federal Circuit, (1) examples are not necessary to support the adequacy of a written description; and (2) the written description standard may be met ... even where actual reduction to practice of an invention is absent. *Falkner v. Inglis*, 448 F.3d 1357, 1366, 79 USPQ2d 1001, 1007 (Fed. Cir. 2006). The Federal Circuit in *LizardTech, Inc. v. Earth Resource Mapping, PTY, Inc.* stated:

> A claim will not be invalidated on section 112 grounds simply because the embodiments of the specification do not contain examples explicitly covering the full scope of the claim language. That is because the patent specification is written for a person of skill in the art, and such a person comes to the patent with the knowledge of what has come before. Placed in that context, it is unnecessary to spell out every detail of the invention in the specification; only enough must be included to convince a person of skill in the art that the inventor possessed the invention and to enable such a person to make and use the invention without undue experimentation.

424 F.3d 1336, 1345 (Fed. Cir. 2005) (citing Union Oil Co. v. Atl. Richfield Co., 208 F.3d 989, 997 (Fed. Cir. 2000); In re GPAC Inc., 57 F.3d 1573, 1579 (Fed. Cir. 1995)).

Claim construction is an essential part of the examination process. Each claim must be separately analyzed and given its broadest reasonable interpretation in light of and consistent with the written description. *See, e.g., In re Morris*, 127 F.3d 1048, 1053-54, 44 USPQ2d 1023, 1027 (Fed. Cir. 1997). The Examiner states that the term "antigen" used in the claims is not limited to the entire intact protein, so there is more than one form of the single naturally occurring protein. In lines 2-4 of page 26 of the specification, it is stated that "...the polypeptide produced for the polypeptide vaccine or by the DNA vaccine can be the entire 16 (±4) kDa antigen and/or 30 (±4) kDa antigen..." According to definitions set out in the present specification:

> The term "antigen" as used herein refers to a substance which stimulates production of antibody or sensitized cells during an immune response. An antigen includes the whole pathogen or *a particular protein of the pathogen*. An antigen consists of multiple epitopes, each epitope of which is capable of causing the production of an antibody against the particular epitope.
>
> The term "epitope" as used herein refers to an immunogenic region of an antigen which is recognized

> by a particular antibody molecule. In general, an antigen will possess one or more epitopes, each capable of binding an antibody that recognizes the particular epitope.

Page 12, lines 5-18 of the specification, emphasis added.

It is clear from this definition, as opposed to the term "polypeptide", that the term "antigen" refers to the *entire* protein from the pathogen, *ie.* whole antigens, not antigenic fragments. The antigen can possess one or more epitopes. While an epitope is only a portion of an antigen, the antigen is the entire protein. Therefore the phrase "a single naturally occurring 16 (±4) kDa protein antigen isolated from *Sarcocystis neurona* and a single naturally occurring 30 (±4) kDa protein antigen isolated from *Sarcocystis neurona*" refer to the entire 16 (±4) kDa and entire 30 (±4) kDa proteins that are naturally occurring in *Sarcocystis neurona*.

Therefore, the claimed subject matter was described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Reversal of the rejection is requested.

(B) The Examiner rejected Claims 4, 13 and 46 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention, since a disclosure cannot teach one to make or use something that has not been described.

The amount of guidance or direction needed to enable the invention is inversely related to the amount of knowledge in the state of the art as well as the predictability in the art. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). The "amount of guidance or direction" refers to that information in the application, as originally filed, that teaches exactly how to make or use the invention. The more that is known in the prior art about the nature of the invention, how to make, and how to use the invention, and the more predictable the art is, the less information needs to be explicitly stated in the specification. Liang *et al.* is provided to illustrate the state of the art at the time of filing. Naturally occurring proteins can be isolated an identified by their molecular weight as shown in Figure 3 on page 1836 of Liang *et al.*,

Infection and Immunity, vol. 66, no. 5, pp. 1834-1838 (1998). Clearly, a person of ordinary skill in the art would be capable of making the composition consisting of the single naturally occurring protein antigens isolated from *Sarcocystis neurona*. While Example 1 is directed to injection of mice, administration of the vaccines to an equid is described on page 13, line 24 through page 15, line 7. Therefore the specification enables one skilled in the art to make and/or use the invention. Reversal of the rejection is requested.

(C) The Examiner rejected Claims 4, 13, 46 and 50 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

The courts have described the essential question to be addressed in a description requirement issue in a variety of ways. An objective standard for determining compliance with the written description requirement is, "does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed." *In re Gosteli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989). Compliance with the written description requirement is a question of fact which must be resolved on a case-by-case basis. *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116 (Fed. Cir. 1991). Generally, there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement. Information which is well known in the art need not be described in detail in the

specification. *See, e.g., Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986). For some biomolecules, examples of identifying characteristics include a sequence, structure, binding affinity, binding specificity, molecular weight, and length. Although structural formulas provide a convenient method of demonstrating possession of specific molecules, other identifying characteristics or combinations of characteristics may demonstrate the requisite possession. Thus, there is no *per se* rule that a protein must be described by its amino acid sequence. Considering the skill in the art of isolating proteins from organisms such as *Sarcocystis neurona*, naturally occurring proteins can be isolated an identified by their molecular weight as shown in Figure 3 on page 1836 of Liang *et al.*, *Infection and Immunity*, vol. 66, no. 5, pp. 1834-1838 (1998).

Possession may be shown in a variety of ways including description of an actual reduction to practice, or by showing that the invention was "ready for patenting" such as by the disclosure of drawings or structural chemical formulas that show that the invention was complete, or by describing *distinguishing identifying characteristics*

sufficient to show that the applicant was in possession of the claimed invention. See, *e.g.*, *Pfaff v. Wells Elecs., Inc.*, 525 U.S. 55, 68, 119 S.Ct. 304, 312, 48 USPQ2d 1641, 1647 (1998) (emphasis added); *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406; *Amgen, Inc. v. Chugai Pharmaceutical*, 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991) (one must define a compound by "whatever characteristics sufficiently distinguish it"). "Compliance with the written description requirement is essentially a fact-based inquiry that will 'necessarily vary depending on the nature of the invention claimed.'" *Enzo Biochem*, 323 F.3d at 963, 63 USPQ2d at 1613.

Possession of a protein does not turn on whether the protein's amino acid sequence is known. Claims directed to DNA were at issue in *In re Wallach.* In regards to the proteins, the Federal Circuit stated:

> *Whether Appellants were in possession of the protein says nothing about whether they were in possession of the protein's amino acid sequence.* Although Appellants correctly point out that a protein's amino acid sequence is an inherent property of the protein, the fact that Appellants may have isolated and thus physically possessed TBP-II does not amount to knowledge of that protein's sequence or possession of any of its other descriptive properties

In re Wallach 378 F.3d 1330, 71 USPQ2d 1939, 1943 (Fed.

Cir. 2004) (emphasis added).

Although the Examiner compares the present fact pattern to *Fiddes*, the presently claimed invention does not attempt to claim a broad class of proteins, across many species, as was attempted in *Fiddes*. The present claims are narrowly limited to protein antigens isolated from the specific organism, *Sarcocystis neurona*. Additionally, in *Noelle v. Lederman*, the Federal Circuit stated: "It should be noted, however, that this court in *Vas-Cath* warned that each case involving the issue of written description, "must be decided on its own facts. Thus, the precedential value of cases in this area is extremely limited." *Noelle v. Lederman* 355 F.3d 1343, 69 USPQ2d 1508, 1513 (Fed. Cir. 2004) citing *Vas-Cath*, 935 F.2d at 1562 (quoting *In re Driscoll*, 562 F.2d 1245, 1250 (C.C.P.A. 1977)). The written description is believed to be adequate without the necessity of providing the amino acid sequence of the proteins comprising the composition. Reversal of the rejection is requested.

B. Conclusion

As shown above, the claimed subject matter is described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed subject matter. Also, the claimed subject matter was described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention. Therefore, Claims 4, 13, 46 and 50 are patentable. Reversal of the Final Rejection is requested.

Respectfully,

Ian C. McLeod
Registration No. 20,931

IAN C. McLEOD, P.C.
2190 Commons Parkway
Okemos, MI 48864

Telephone: (517) 347-4100
Facsimile: (517) 347-4103

CLAIMS APPENDIX

4. A composition consisting of a single naturally occurring 16 (±4) kDa protein antigen isolated from *Sarcocystis neurona* and a single naturally occurring 30 (±4) kDa protein antigen isolated from *Sarcocystis neurona* in a pharmaceutically accepted carrier.

13. A method for treating an equine with a *Sarcocystis neurona* infection comprising:

(a) providing a composition consisting of a single naturally occurring 16 (±4) kDa protein antigen isolated from *Sarcocystis neurona* and a single naturally occurring 30 (±4) kDa protein antigen isolated from *Sarcocystis neurona* in a pharmaceutically accepted carrier; and

(b) inoculating the equine with the composition to treat the equine with the *Sarcocystis neurona* infection.

46. A method for treating a disease in an equine caused by a *Sarcocystis neurona* infection which comprises providing a composition which when injected into the equine causes the equine to produce antibodies against a 16 (±4) kDa antigen and a 30 (±4) kDa antigen of the *Sarcocystis neurona* which treats the disease caused by the *Sarcocystis neurona,* wherein the composition consists of a single naturally occurring 16 (±4) kDa protein antigen isolated from *Sarcocystis neurona* and a single naturally occurring 30 (±4) kDa protein antigen isolated from *Sarcocystis neurona* in a pharmaceutically accepted carrier.

50. The method of Claim '46 wherein the composition is administered by an inoculation route selected from the group consisting of intranasal administration, intramuscular injection, intraperitoneal injection, intradermal injection, and subcutaneous injection.

EVIDENCE APPENDIX

Attached are copies of cited patents and publications:

1. Liang *et al.*, *Infection and Immunity*, vol. 66, no. 5, pp. 1834-1838 (1998).

RELATED PROCEEDINGS APPENDIX

Attached are copies of the following decisions:

1. Decision by the Board for Application No. 09/670,355 ('355).

2. Decision by the Board for Application No. 09/670,096 ('096).

3. Decision by the Board for Application No. 09/669,843 ('843).

INFECTION AND IMMUNITY, May 1998, p. 1834–1838
0019-9567/98/$04.00+0

Vol. 66, No. 5

Evidence that Surface Proteins Sn14 and Sn16 of *Sarcocystis neurona* Merozoites Are Involved in Infection and Immunity†

FANG TING LIANG, DAVID E. GRANSTROM, XIAO MIN ZHAO, AND JOHN F. TIMONEY*

Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, Kentucky 40546

Received 7 July 1997/Returned for modification 26 August 1997/Accepted 29 January 1998

***Sarcocystis neurona* is the etiologic agent of equine protozoal myeloencephalitis (EPM). Based on an analysis of 25,000 equine serum and cerebrospinal fluid (CSF) samples, including samples from horses with neurologic signs typical of EPM or with histologically or parasitologically confirmed EPM, four major immunoblot band patterns have been identified. Twenty-three serum and CSF samples representing each of the four immunoblot patterns were selected from 220 samples from horses with neurologic signs resembling EPM and examined for inhibitory effects on the infectivity of *S. neurona* by an in vitro neutralization assay. A high correlation between immunoblot band pattern and neutralizing activity was detected. Two proteins, Sn14 and Sn16 (14 and 16 kDa, respectively), appeared to be important for in vitro infection. A combination of the results of surface protein labeling, immunoprecipitation, Western blotting, and trypsin digestion suggests that these molecules are surface proteins and may be useful components of a vaccine against *S. neurona* infection. Although *S. neurona* is an obligate intracellular parasite, it is potentially a target for specific antibodies which may lyse merozoites via complement or inhibit their attachment and penetration to host cells.**

The apicomplexan *Sarcocystis neurona* is the causative agent of equine protozoal myeloencephalitis (14), a progressive disease affecting the central nervous system (4, 7, 13). Cases of equine protozoal myeloencephalitis (EPM) have been reported among native horses in North, Central, and South America (3, 10, 11, 15, 16, 26). Serological testing based on immunoblot patterns in Kentucky, Ohio, Pennsylvania, and Oregon detected an average *S. neurona* exposure rate of 45% (5, 6, 18, 32). The New York State Veterinary College at Cornell University reported that 25% of equine neurologic disease accessions were due to EPM in 1978 (19). The number of cases diagnosed at necropsy at the Livestock Disease Diagnostic Center at the University of Kentucky increased from approximately 8% of all neurological accessions during 1988 to 1990 to 15% during 1991 to 1992 (19).

Although no successful vaccine against related apicomplexan parasites has been widely used, there are encouraging signs that such a vaccine is possible. Surface antigens of coccidia have been shown to be involved in interactions with the host cell membrane during invasion (9, 24), and apical complex proteins of some coccidia have been found to be targets of protective antibodies (24, 28, 33, 34). Apical complex organelles of sporozoites appear to secrete their contents during host cell attachment and formation of the parasitophorus vacuole (2, 30, 35).

Although the pathogenesis of EPM is not fully known, the following events are believed to occur. *S. neurona* sporozoites penetrate the horse's intestinal tract, enter vascular endothelial cells, and complete at least one merogonous generation. As immune responses, including antibody production are induced, merozoites may pass through the vascular endothelium of the blood-brain barrier into the immune privileged central nervous system, where they survive. The high rate of exposure to *S. neurona* and the relatively low incidence of clinical EPM indicate that most horses develop effective immunity that may prevent entry into the central nervous system (5, 6, 18, 32).

Since 1991, approximately 25,000 equine serum and cerebrospinal fluid (CSF) samples, including samples from horses with neurologic signs and with histologically or parasitologically confirmed EPM, have been tested for specific antibody to *S. neurona* at the University of Kentucky. Four immunoblot band patterns could be consistently identified in these samples. The objective of this study was to attempt to correlate immunoblot band patterns with in vitro neutralizing activity of the serum and CSF. Twenty-three serum and CSF samples, each from a different horse and representative of each of the four band patterns, were selected from a set of samples from 220 horses with a clinical diagnosis of a neurologic disorder resembling EPM and tested for inhibitory activities on parasite infection by an in vitro neutralization assay. Antibodies to two surface polypeptides were correlated with in vitro neutralizing activity.

MATERIALS AND METHODS

Parasite. *S. neurona* SN3 was originally isolated from the spinal cord of a horse with histologically confirmed EPM (16).

Cell and tissue culture medium. Bovine turbinate (BT) cells were purchased from the American Type Culture Collection (Rockville, Md.). Cells were seeded in 75- or 25-cm^2 tissue culture flasks (Corning Inc., Corning, N.Y.) and incubated in an atmosphere containing 5% CO_2 and 95% air at 37°C. The cell culture was maintained in RPMI 1640 supplemented with 15% fetal calf serum (FCS), 2 mM sodium pyruvate, 0.075% (wt/vol) sodium bicarbonate, 120 U of penicillin per ml, and 120 μg of streptomycin (BioWhittaker, Walkersville, Md.) per ml. Subconfluent cell culture was used in all of the experiments.

Clinical samples of serum and CSF. Twenty-three serum and CSF samples from different horses were selected to represent each immunoblot pattern from a group of samples from 220 horses with a clinical diagnosis of a neurologic disorder resembling EPM. These samples were originally submitted to our laboratory for serological testing for EPM from throughout the United States.

Immunoblotting. Immunoblotting was performed as previously described (17). Approximately 1.5×10^7 *S. neurona* merozoites were harvested from BT cell culture and dissolved in an appropriate volume of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) sample buffer (65 mM Tris, 2% SDS, 10% glycerol, 1.5% 2-mercaptoethanol [pH 6.8]). After heating in a boiling water bath for 5 min, the sample was separated in an SDS–10 to 20% linear gradient

* Corresponding author. Mailing address: Department of Veterinary Science, 108 Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546-0099. Phone: (606) 257-4172. Fax: (606) 257-6169. E-mail: jtimoney@pop.uky.edu.

† Contribution no. 97-14-91 from the Kentucky Agricultural Experiment Station, with approval of the director.

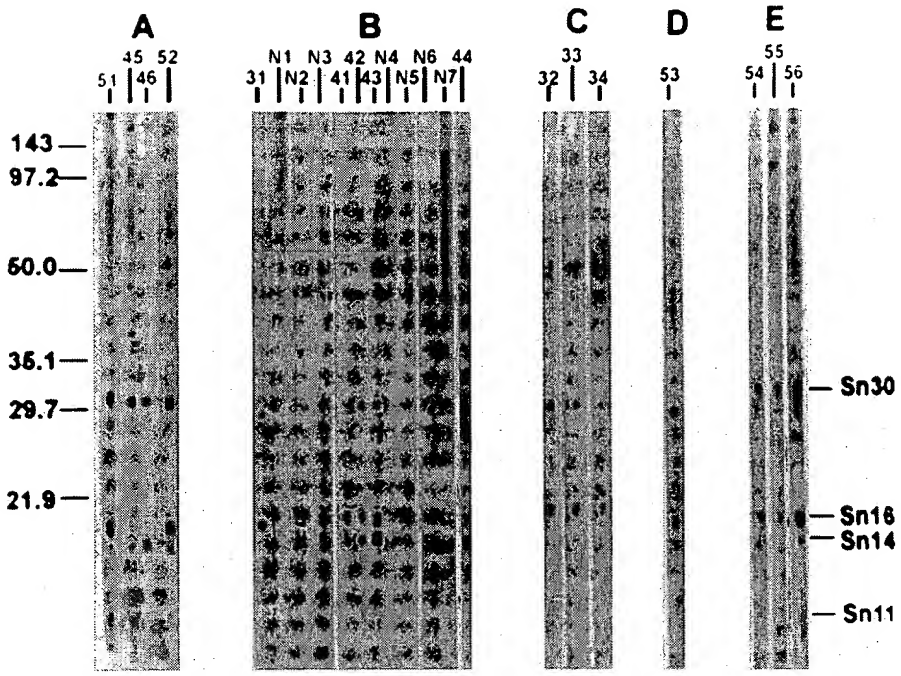

FIG. 1. Immunoblot patterns of proteins of *S. neurona* following reaction with equine serum and CSF samples. The five panels represent samples selected from each of five immunoblots of 45 serum and CSF samples performed in a Miniblotter 45. N4, N5, 34, and 51 were CSF samples. The numbers at the left are molecular masses (in kilodaltons).

polyacrylamide gel with a thickness of 0.75 mm, using a discontinuous buffer system (25). Separated proteins were electrotransferred to nitrocellulose (NC; Costar Co., Cambridge, Mass.) in Towbin transfer buffer (37). The blot was blocked in 5% nonfat dry milk and 0.4% Tween 20 in phosphate-buffered saline (PBS; pH, 7.2) and then placed in a Miniblotter 45 (Immunetics, Cambridge, Mass.). Serum or CSF diluted 1:10 or 1:2, respectively, with blocking solution, was applied. Biotinylated goat anti-horse immunoglobulin G, followed by streptavidin-peroxidase conjugate (Pierce, Rockford, Ill.) and aminoethyl carbazole-hydrogen peroxide, was used to develop the blot.

Neutralization assay. Serum and CSF samples were filtered through 0.22-μm-pore-size syringe filters (Micron Separations Inc., Westborough, Mass.). FCS was used as a control. Approximately 3.3×10^6 *S. neurona* merozoites freshly isolated from BT cell culture were resuspended in 1.0 ml of serum or CSF and incubated at 37°C for 60 min with occasional shaking. The merozoites were then pelleted by centrifugation at 300 × *g* for 5 min at 37°C. Each of the treated merozoite samples was seeded into three 25-cm^2 tissue culture flasks. Two days postinfection, merozoites remaining in the medium were removed and fresh medium was added. On day 5, schizonts in 10 fields (400×) of each flask were counted under a phase-contrast microscope. Merozoites were recovered and counted in a hemacytometer on day 7.

Surface protein labeling. About 8×10^7 merozoites freshly harvested from BT culture were washed twice with excess volumes of Na_2CO_3 buffer (50 mM Na_2CO_3, 0.85% NaCl [pH 7.4]) by centrifugation at 300 × *g* for 10 min at 37°C. The organisms were then resuspended in 1 ml of Na_2CO_3 buffer containing 100 μg ImmunoPure Sulfo-NHS-Biotin (Pierce), incubated at 37°C for 10 min, and then washed twice in excess volumes of Na_2CO_3 buffer at 4°C. The biotin-labeled merozoites were lysed in 1 ml of lysis buffer (50 mM Tris, 1% Triton X-100, 0.1% SDS, 1 mM EDTA [pH 7.6]) at 4°C for 30 min. The lysate was collected by centrifugation at 3,000 × *g* for 30 min at 4°C.

Immunoprecipitation. Preparations for immunoprecipitation were conducted at 4°C. Aliquots of lysate from 8×10^7 biotin-labeled merozoites were mixed with 150 μl of positive serum from horse with a histologically diagnosed case of EPM and with serum from a normal horse and incubated for 30 min. The mixtures were each incubated with 150 μl of GammaBind Plus Sepharose gel (Pharmacia LKB Biotechnology, Uppsala, Sweden) for an additional 30 min. The gels were washed twice with excess volumes of lysis buffer and once with PBS-Tween 20 (0.4% Tween 20 in PBS [pH 7.4]). Finally, the gels were mixed with 200 μl of SDS-PAGE sample buffer, heated in a boiling water bath for 5 min, and centrifuged at 3,000 × *g* for 30 min.

Western blotting. Approximately 3×10^5 biotin-labeled or unlabeled merozoites dissolved in SDS-PAGE sample buffer were loaded in each lane of an SDS–10 to 20% gradient polyacrylamide gel with a thickness of 0.75 mm, or 60 μl of immunoprecipitated antigen was loaded in each lane of a 1.5-mm 10 to 20% gradient gel. Resolved biotin-labeled proteins were electroblotted to NC, while unlabeled proteins were electrotransferred to a polyvinylidene difluoride membrane (PVDF; NEN Research Products, Boston, Mass.). The NC blots were developed as described for immunoblotting, but the antibody step was omitted. The PVDF blots were stained with Coomassie blue.

Trypsin digestion. Approximately 2×10^7 freshly harvested merozoites were washed twice in RPMI 1640 and then resuspended in 2 ml of trypsin-EDTA (BioWhittaker) at 37°C. Three hundred microliters of a digest was removed at 1 and 5 min after digestion. The digests were mixed with 1.2 ml of prechilled FCS to stop the trypsin reaction and centrifuged at 4,000 × *g* for 5 min at 4°C. After three washes with prechilled 50 mM Tris-HCl buffer (pH 7.6), intact merozoites were counted and dissolved in SDS-PAGE sample buffer. An extract of 6×10^4 merozoites/μl was prepared for immunoblotting.

Statistical analysis. PROC GLM of SAS was used to analyze the data (SAS Institute Inc., Cary, N.C.). The *F* test and least significant difference procedure were used to compare the mean values of assay results. A *P* value of <0.05 was taken as a significant difference.

RESULTS

Immunoblot band patterns. The four immunoblot band patterns based on combinations of the Sn30, -16, -14, and -11 proteins (30, 16, 14, and 11 kDa, respectively) are presented (Fig. 1). Nineteen serum and four CSF samples selected from 220 clinical samples were grouped according to their band patterns (Fig. 1 and 2): group 1, N1 to N7; group 2, 31 to 34; group 3, 41 to 46; and group 4, 51 to 56. Serum or CSF samples of group 1 were not reactive with Sn16, Sn14, or Sn11; sera from groups 2, 3, and 4 consistently reacted with Sn16, Sn14, and Sn11, respectively.

Correlation of band patterns with inhibitory activities. The results of neutralization assays are presented in Fig. 2. As expected, serum or CSF samples of the same group showed similar neutralizing activities. Serum and CSF samples of groups 2 and 4 showed approximately equal inhibitory activities, while group 3 sera showed the greatest inhibitory activity of the four

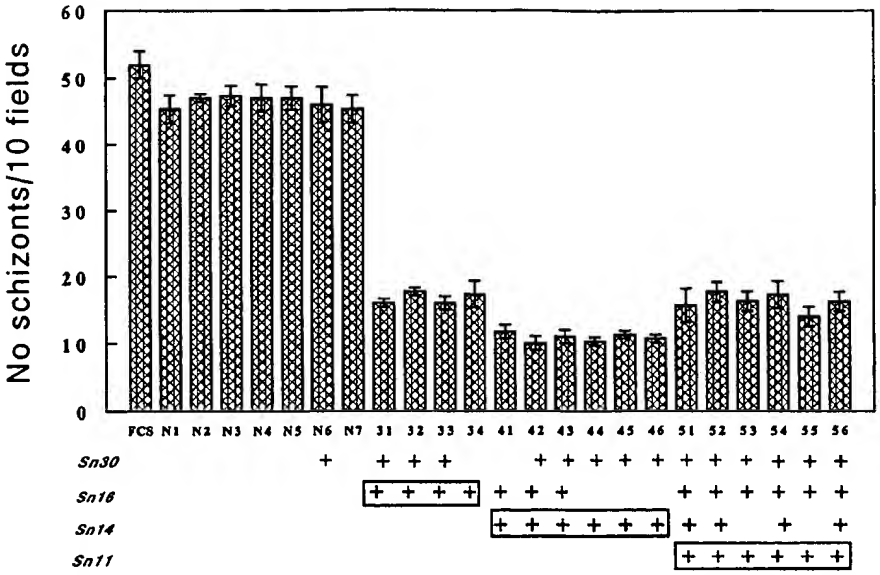

FIG. 2. Neutralization of *S. neurona* infectivity by serum and CSF samples. Merozoites were incubated in serum or CSF samples for 60 min at 37°C. FCS was used as a control. On day 5 postinfection, schizonts in 10 fields (400×) were counted for each flask. Means and standard deviations are presented. Bands recognized by both serum and CSF are shown at the bottom.

groups. Based on these observations, it appears that Sn16 and Sn14 may have important roles during the initial stage of *S. neurona* infection and that antibody to Sn14 may be more effective in neutralization than antibody to Sn16. No inhibitory activity correlating with antibody to Sn30 was noted. Interestingly, group 4 sera that contained antibody to both Sn14 and Sn11 had neutralizing activity similar to that of group 2, suggesting that Sn11 antibody in serum may block the neutralizing effect of Sn14 antibody (Fig. 1 and 2).

Surface protein labeling and immunoprecipitation. A combination of surface protein labeling, immunoprecipitation, and Western blotting was conducted to determine whether Sn16 and Sn14 are surface proteins. Proteins similar in size to these two proteins were labeled (Fig. 3A, lane b), a result confirmed by immunoprecipitation (Fig. 3A, lane c). Negative serum did not precipitate any *Sarcocystis* protein (Fig. 3A, lane d).

Effect of trypsin digestion. The rapid action of trypsin suggests that these proteins were very accessible to the action of the enzyme and therefore on the cell surface. Within 1 min the Sn14 band was no longer visible, and the Sn16 band showed significantly reduced density at 5 min (Fig. 3B). The density of the Sn30 band was also reduced after 5 min. The trypsin-resistant band between Sn16 and Sn14 in Fig. 3B was recognized by only few equine sera and was apparently not a surface protein, as determined by surface labeling and immunoprecipitation (Fig. 3A). Since trypsin digestion could lyse the parasite, digestion was monitored by counting intact merozoite cells. A significant reduction in merozoite numbers was not observed until after trypsin digestion for 40 min.

DISCUSSION

Humoral immunity may play an essential role in clearing *S. neurona* merozoites at the extracellular stage. Specific antibodies may lyse the merozoites via complement, inhibit their infection by blocking attachment and penetration, or bind to surface receptors and disorder signal transductions. These results suggest that sera containing antibodies specific for Sn16 and Sn14 reduce parasite infection, probably by binding to the merozoite cell surface and blocking attachment and/or penetration. An extensive body of data is available to indicate that antibody to apicomplexan parasites is protective. Treatment of *Cryptosporidium*-infected immunocompromised patients with hyperimmune bovine colostrum has ameliorated or eliminated clinical symptoms (38), an effect correlated with antibodies specific for sporozoite surface proteins (12, 29, 36). Invasion of

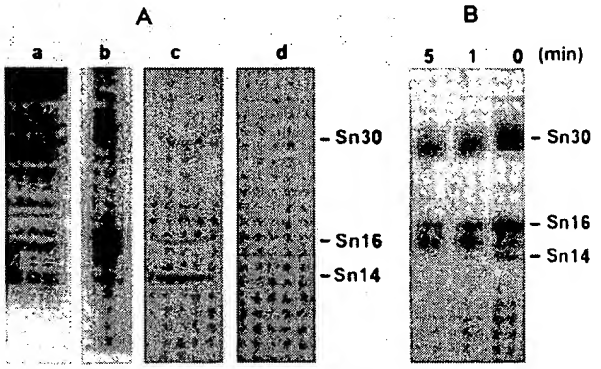

FIG. 3. Evidence for surface localization of Sn14 and Sn16. (A) Analysis of surface proteins by labeling, immunoprecipitation, and Western blotting. Lane a, proteins in an extract of unlabeled merozoites following SDS-PAGE and electrotransfer to PVDF (Coomassie blue stain). Lane b, proteins in an extract of biotin-labeled merozoites following SDS-PAGE and electrotransfer to NC. The blot was probed with peroxidase-streptavidin conjugate. Lane c, biotin-labeled proteins in a lysate of biotin-labeled merozoites immunoprecipitated with serum from a horse with a histologically confirmed case of EPM. Precipitated proteins were separated by SDS-PAGE and electrotransferred to NC. The blot was developed as described for lane b. Lane d, band pattern of biotin-labeled proteins in a lysate of biotin-labeled merozoites immunoprecipitated with EPM-negative serum and developed as described for lane c. (B) Trypsin digestion. Merozoites were digested by trypsin for 1 and 5 min at 37°C followed by SDS-PAGE and electrotransfer to NC. The blot was treated with serum from a horse with a histologically confirmed case of EPM.

target cells by trypomastigotes of *Trypanosoma cruzi* is receptor mediated and can be blocked by specific antibodies (1, 39). Yet another example is the penetration-enhancing factor of *Toxoplasma gondii* that has been identified by using monoclonal antibodies (34).

Detection of *S. neurona* infection by demonstration of reactivity of serum and CSF samples with the Sn11, Sn14, and Sn16 antigens has been extensively used as a diagnostic tool (5, 6, 32) and is sensitive (20, 21). However, the test has not yet been fully validated by studies of serum and CSF samples from cases in which *S. neurona*-like organisms have been detected histologically and cultured. Neutralization assays revealing significant differences in inhibitory activities between the groups of serum and CSF samples with different immunoblot band patterns strongly suggest that antibodies specific for Sn14 and Sn16 have protective activity against *S. neurona*, at least in vitro (Fig. 1 and 2) and support the use of the immunoblot test in diagnosis of EPM. Antibodies to Sn30 are not recognized as specific since a 30-kDa antigen immunoreactive with sera from horses with EPM is found in other *Sarcocystis* spp.

The serum neutralization data obtained in this study were based mainly on the use of an in vitro bioassay developed in our laboratory. Although assays for other apicomplexan parasites such as *Cryptosporidium* (12), *Plasmodium* (24), and *Toxoplasma* (27) species have been described, this study represents the first application of such an assay to a *Sarcocystis* sp. Optimum inhibition required sensitization of merozoites in serum or CSF for at least 40 min (data not shown), suggesting that maximum inhibition of parasite infection requires saturation with specific antibody. Although serum and CSF samples of the same band pattern group did not have equal antibody activities as estimated by immunoblotting (Fig. 1), all samples saturated their antigens under the assay conditions and gave similar inhibitions in neutralization assays (Fig. 2). This result was supported by serum dilution and time of incubation data (data not shown). Although schizont and merozoite counts under conditions of maximum inhibition were not equal for these two experiments, percent reductions in numbers of schizonts and merozoites were very similar, i.e., 84 and 92% in the serum dilution assay, compared with 81 and 84% in the incubation assay. These small differences in counts resulted from the unequal dosages of merozoites used for infection in the two assays. Reductions in schizont production by group 3 sera ranged from 78% (sample 45) to 80% (sample 42) relative to the FCS control (Fig. 2). These highly consistent results suggest that the assay was valid and that counts of schizonts and merozoites can serve as indicators of inhibitory activity.

Although *S. neurona* was sensitive to specific antibodies, a 10-min exposure to antiserum was required to yield a significant reduction in parasite production (data not shown). This may partially explain why protective antibodies to some apicomplexan parasites are effective in vitro but not in vivo (23). Newly released parasites are exposed to serum for a shorter time in vivo, and the access of neutralization-sensitive epitopes to antibody may be limited (31). Merozoites in vivo may move more directly from cell to cell. However, in the case of EPM, disease occurs only after the merozoite passes through the vascular endothelium of the blood-brain barrier into the central nervous system, and so humoral responses may play an essential role in blocking this migration. Moreover, specific cytotoxic T cells are ineffective in attacking merozoites migrating to the central nerve system in the bloodstream.

When antibodies to Sn11 and Sn14 (group 4) coexisted, the inhibition activity of the serum was reduced to that of sera of group 2 (Fig. 2), suggesting that antibody to Sn11 blocked the effect of Sn14 antibody. Therefore, these two proteins may be located in close proximity on the merozoite surface.

The results of biotin labeling and immunoprecipitation studies (Fig. 3A) are consistent with the hypothesis that the effects of antibodies to Sn14 and Sn16 are mediated via binding to surface antigens. A combination of these techniques has been shown to be effective in the identification of specific surface antigens (8). However, since nonantigenic proteins may be coprecipitated, the results may not be definitive. The results of controlled trypsin digestion were, however, consistent with the conclusion that Sn14 and Sn16 are localized on the surface of the parasite (Fig. 3).

Although monoclonal antibodies are often used to study parasitic proteins, the sera of naturally infected animals have unique advantages in that they can provide important information on protectively immunogenic proteins in the natural host. The parasite may express different proteins at different stages of in vivo or in vitro development; and some proteins may be expressed and function essentially only in vitro. Such proteins would be inappropriate targets for vaccine development. *S. neurona* infection of the horse induces production of antibodies to Sn16 and Sn14, indicating that these two proteins are expressed in vivo and are strong immunogens in the horse. Clearly, they warrant further investigation as candidate antigens for inclusion in vaccines against *S. neurona* infection.

ACKNOWLEDGMENTS

This work was supported in part by grants from the Grayson-Jockey Club Research Foundation and the Abercrombie Foundation.

REFERENCES

1. **Alvers, M. J., G. Abuin, V. Y. Kuwajima, and W. Colli.** 1986. Partial inhibition of trypomastigote entry into cultured mammalian cells by monoclonal antibodies against a surface glycoprotein of *Trypanosoma cruzi*. Mol. Biochem. Parasitol. **21**:75–82.
2. **Bannister, L. H., G. H. Mitchell, G. A. Butcher, and E. D. Dennis.** 1986. Lamellar membranes associated with rhoptries in erythrocytic merozoites of *Plasmodium knowlesi*: a clue to the mechanism of invasion. Parasitol. **92**: 291–303.
3. **Barros, C. S. L., S. S. Barros, and M. N. Santos.** 1986. Equine protozoal myeloencephalitis in south Brazil. Vet. Rec. **119**:283–284.
4. **Beech, J., and D. C. Dodd.** 1974. *Toxoplasma*-like encephalitis in the horse. Vet. Pathol. **11**:87–96.
5. **Bentz, B. G., D. E. Granstrom, and S. Stamper.** 1997. Seroprevalence of antibodies to *Sarcocystis neurona* in horses residing in a county of southeastern Pennsylvania. J. Am. Vet. Med. Assoc. **210**:517–518.
6. **Blythe, L. L., D. E. Granstrom, D. E. Hansen, L. L. Walker, J. Bartlett, and S. Stamper.** 1997. Seroprevalence of antibody to *Sarcocystis neurona* in horses residing in Oregon. J. Am. Vet. Med. Assoc. **210**:525–527.
7. **Boy, M. G., D. T. Galligan, and T. J. Divers.** 1990. Protozoal encephalomyelitis in horses: 82 cases (1972–1986). J. Am. Vet. Med. Assoc. **196**:632–634.
8. **Cole, S. R., L. K. Ashman, and P. L. Ey.** 1987. Biotinylation: an alternative to radioiodination for the identification of cell surface antigens in immunoprecipitates. Mol. Immunol. **24**:699–705.
9. **Coppel, R. L.** 1992. Repeat structures in a *Plasmodium falciparum* protein (MESA) that binds human erythrocyte protein 4.1. Mol. Biochem. Parasitol. **50**:335–348.
10. **Davis, S. W., B. M. Daft, and J. P. Dubey.** 1991. *Sarcocystis neurona* cultured *in vitro* from a horse with equine protozoal myelitis. Equine Vet. J. **23**: 315–317.
11. **Davis, S. W., C. A. Speer, and J. P. Dubey.** 1991. *In vitro* cultivation of *Sarcocystis neurona* from the spinal cord of a horse with equine protozoal myelitis. J. Parasitol. **77**:789–792.
12. **Doyle, P. S., J. Crabb, and C. Petersen.** 1993. Anti-*Crytosporidium parvum* antibodies inhibit infectivity in vitro and in vivo. Infect. Immun. **61**:4079–4084.
13. **Dubey, J. P., S. W. Davis, A. Koestner, and K. Kiryu.** 1974. Equine encephalomyelitis due to a protozoan parasite resembling *Toxoplasm gondii*. J. Am. Vet. Med. Assoc. **165**:249–255.
14. **Dubey, J. P., S. W. Davis, and C. A. Speer.** 1991. *Sarcocystis neurona* n.sp. (Protozoa: Apicomplexa), the etiologic agent of equine protozoal myeloencephalitis. J. Parasitol. **77**:212–218.
15. **Fayer, R., I. G. Mayhew, J. D. Baird, S. G. Dill, J. H. Foreman, J. C. Fox, I. J. Higgins, S. M. Reed, W. W. Ruoff, R. W. Sweeney, and P. Tuttle.** 1990.

Epidemiology of equine protozoal myeloencephalitis in North America based on histologically confirmed cases. J. Vet. Int. Med. **4**:54–57.
16. **Granstrom, D. E., O. Alvarez, Jr., P. F. Comer, and J. P. Dubey.** 1992. Equine protozoal myeloencephalitis in a native Panamanian horse and isolation of *Sarcocystis neurona*. J. Parasitol. **78**:909–912.
17. **Granstrom, D. E., J. P. Dubey, S. W. Davis, R. Fayer, J. C. Fox, K. B. Poonacha, R. C. Giles, and F. Comer.** 1993. Equine protozoal myeloencephalitis: Antigen analysis of cultured *Sarcocystis neurona* merozoites. J. Vet. Diagn. Invest. **5**:88–90.
18. **Granstrom, D. E., and S. M. Reed.** 1994. Equine protozoal myeloencephalitis. Equine Pract. **16**:23–26.
19. **Granstrom, D. E., J. P. Dubey, R. C. Giles, A. A. Gajadhar, C. K. Fenger, et al.** 1994. Equine protozoal myeloencephalitis: biology and epidemiology, p. 109–111. *In* H. Nakajima and W. Plowright (ed.), Proceeding, 7th International Conference on Equine Infectious Diseases 1994, Tokyo, Japan. R&W Publications Ltd., Newmarket, England.
20. **Granstrom, D. E.** 1995. Equine protozoal myeloencephalitis testing: review of 1993 and 1994. Proc. Am. Assoc. Equine Pract. **41**:218–219.
21. **Granstrom, D. E.** 1995. Recent advances in the laboratory diagnosis of equine parasitic diseases. Vet. Clin. North Am. Equine Pract. **11**:437–442.
22. **Hamir, A. N., G. Moser, D. T. Galligan, S. W. Davis, D. E. Granstrom, and J. P. Dubey.** 1993. Immunohistochemical study to demonstrate *Sarcocystis neurona* in equine protozoal myeloencephalitis. J. Vet. Diagn. Invest. **5**:88–90.
23. **Hines, S. A., G. H. Palmer, D. P. Jasmer, W. L. Goff, and T. F. McElwain.** 1995. Immunization of cattle with recombinant *Babesia bovis* merozoite surface antigen-1. Infect. Immun. **63**:349–352.
24. **Hollingdale, M. R., E. H. Nardin, S. Tharavanij, A. L. Schwartz, and R. S. Nussenzweig.** 1984. Inhibition of entry of *Plasmodium falciparum* and *P. vivax* sporozoites into cultured cells: an *in vitro* assay of protective antibodies. J. Immunol. **132**:909–913.
25. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**:680–685.
26. **Masri, M. D., J. Lopez de Alda, and J. P. Dubey.** 1992. *Sarcocystis neurona*-associated ataxia in horses in Brazil. Vet. Parasitol. **44**:311–314.
27. **Mineo, J. R., R. McLeod, D. Mack, J. Smith, I. A. Khan, K. H. Ely, and L. H. Kasper.** 1993. Antibodies to *Toxoplasma gondii* major surface protein (SAG-1, P30) inhibit infection of host cells and are produced in murine intestine after peroral infection. J. Immunol. **150**:3951–3964.
28. **Perkins, M. E.** 1992. Rhoptry organelles of Apicomplexan parasites. Parasitol. Today **8**:28–32.
29. **Petersen, C., J. Gut, P. S. Doyle, J. H. Crabb, R. G. Nelson, and J. H. Leech.** 1992. Characterization of an M_r->900,000 *Cryptosporidium parvum* sporozoites glycoprotein recognized by hyperimmune bovine colostral immunoglobulin. Infect. Immun. **60**:5132–5138.
30. **Sam-Yellowe, T. Y., H. Shio, and M. E. Perkins.** 1988. Secretion of *Plasmodium falciparum* rhoptry protein into the plasma membrane of host erythrocytes. J. Cell Biol. **106**:1507–1513.
31. **Saul, A.** 1987. Kinetic constraints on the development of a malaria vaccine. Parasite Immunol. **9**:1–9.
32. **Saville, W. J., S. M. Reed, D. E. Granstrom, K. W. Hinchcliff, C. W. Kohn, et al.** 1997. Seroprevalence of antibody to *Sarcocystis neurona* in horses residing in Ohio. J. Am. Vet. Med. Assoc. **210**:519–524.
33. **Schofield, L., G. R. Bushell, J. A. Cooper, A. J. Saul, J. A. Upcroft, and C. Kidsin.** 1987. A rhoptry antigen of *Plasmodium falciparum* contains conserved and variable epitopes recognized by inhibitory monoclonal antibodies. Mol. Biochem. Parasitol. **18**:183–195.
34. **Schwartzman, J. D.** 1986. Inhibition of a penetration-enhancing factor of *Toxoplasma gondii* by monoclonal antibodies specific for rhoptries. Infect. Immun. **51**:760–764.
35. **Stewart, M. J., S. Schulman, and J. P. Vanderberg.** 1986. Rhoptry secretion of membranous whorls by *Plasmodium falciparum* merozoites. Am. J. Trop. Med. Hyg. **35**:37–44.
36. **Tilley, M., R. Fayer, A. Guidry, S. J. Upton, and B. L. Blagburn.** 1990. *Cryptosporidium parvum* (Apicomplexa: Cryptosporidiidae) oocyst and sporozoite antigens recognized by bovine colostral antibodies. Infect. Immun. **58**: 2966–2971.
37. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
38. **Tzipori, S., D. Robertson, D. A. Cooper, and L. White.** 1987. Chronic cryptosporidiosis and hyperimmune bovine clostrum. Lancet **ii**:344–345.
39. **Yoshida, N., R. A. Mortara, M. F. Araguth, J. C. Gonzalez, and M. Russo.** 1989. Metacyclic neutralizing effect of monoclonal antibody 10D8 directed to the 35- and 50-kilodalton surface glycoconjugates of *Trypanosoma cruzi*. Infect. Immun. **57**:1663–1667.

Editor: S. H. E. Kaufmann

Paper No. 13

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte LINDA S. MANSFIELD, MARY G. ROSSANO, ALICE J. MURPHY and RUTH VRABLE

Appeal No. 2003-1919
Application No. 09/670,355

Ian C. McLeod

ON BRIEF

Before WILLIAM F. SMITH, GRIMES, and GREEN, Administrative Patent Judges.

GREEN, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 10-12, 18-20, 44, 45 and 47-52. Claims 10 and 51 are representative of the subject matter on appeal, and read as follows:

> 10. A vaccine for protecting an equid from a Sarcocystis neurona infection comprising a DNA from Sarcocystis neurona that encodes at least a 16 ± 4 kDa antigen and/or 30 ± 4 kDa antigen of Sarcocystis neurona.

Co: J.

51. A vaccine composition which comprises an effective immunizing amount of DNA derived from Sarcocystis neurona capable of inducing an antibody immune response, and a pharmacologically acceptable carrier.

Claims 10-12, 18-20, 44, 45 and 47-52 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention, i.e., lack of adequate written description. In addition, claims 10-12, 18-20, 44-45 and 47-52 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter that was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention, i.e., lack of enablement. Finally, claims 51 and 52 stand rejected under 35 U.S.C. § 112, second paragraph. After careful review of the record and consideration of the issues before us, we affirm the rejection of claims 10-12, 18-20, 44-45 and 47-52 under 35 U.S.C. § 112, first paragraph, for lack of adequate written description, and the rejection of claims 51 and 52 under 35 U.S.C. § 112, second paragraph, and decline to reach the merits of the rejection of claims 10-12, 18-20, 44-45 and 47-52 under 35 U.S.C. § 112, first paragraph, for lack enablement.

DISCUSSION

1. Rejection under 35 U.S.C. § 112, first paragraph, written description

Claims 10-12, 1-20, 44, 45 and 47-52 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention, i.e., lack of adequate written description.

According to the rejection, "[r]eview of the present specification, the art of record, and a search of the sequence databases for polynucleotides and/or polypeptide sequences of 16(±4) kD antigen and the 30(±4) kD antigen indicate that these sequences have not been identified nor described." Examiner's Answer, page 4. The rejection further contends "the limitation 'at least' in the claims does not limit the invention to 16(±4) kD and/or 30(±4) kD antigen of S. neurona and broadly reads on any antigen that is not disclosed. The specification describes general methods of cloning cDNA sequences from expression libraries; however, the sequences obtained by this method for 16(±4) kD and/or 30(±4) kD antigen are not disclosed." Id. at 4-5. The rejection concludes that "the claimed invention as a whole is not adequately described and is not conventional in the art as of Appellants' effective filing date." Id. at 5 (emphasis in original).

With respect to the issue of conception in the context of an interference count, the Court of Appeals for the Federal Circuit, our reviewing court, has stated that "irrespective of the complexity or simplicity of the method of isolation employed, conception of a DNA, like conception of any chemical substance requires a definition of that substance other than by its functional utility." Fiers v. Revel, 984 F.2d 1164, 1169, 25 USPQ2d 1601, 1604 (Fed. Cir. 1993). The court specifically rejected Fiers' argument "that the existence of a workable method for preparing a DNA establishes conception of that material." Id.

In Enzo Biochem, Inc. v. Gen-Probe Inc., 296 F.3d 1316, 63 USPQ2d 1602 (Fed. Cir. 2002), in determining whether or not a claim to a nucleotide sequence met the written description requirement, the court adopted a portion of the Guidelines proffered by the United States Patent and Trademark Office (USPTO). The court stated that:

> The written description requirement can be met by "showing that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics . . . i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of characteristics.

Enzo Biochem, 296 F.3d at 1324, 63 USPQ2d at 1613 (citations omitted).

In construing the above requirement, the court in In re Wallach, 378 F.3d 1330, 71 USPQ2d 1939 (Fed. Cir. 2004), recognized "that the written description requirement can in some cases be satisfied by functional description." Id., 378

F.3d at 1335. The court held, however, that

> such functional description can be sufficient only if there is also a structure-function relationship known to those of ordinary skill in the art. As we explained above, such a well-known relationship exists between a nucleic acid molecule's structure and its function in encoding a particular amino acid sequence: Given the amino acid sequence, one can determine the chemical structure of all nucleic acid molecules that can serve the function of encoding that sequence. Without that sequence, however, or with only a partial sequence, those structures cannot be determined and the written description requirement is consequently not met.

Id.

In the instant case, as noted by the rejection, neither the disclosure as filed, nor the prior art, discloses any sequence, either amino acid or nucleic, for either the 16(±4) kD and/or 30(±4) kD antigens. Consequently, the written description requirement is not met, and the rejection is affirmed.

Appellants argue with respect to the rejection of claims 10-12, 18-20, 44, 45 and 47-50 that they had "possession of Sarcocystis neurona which contains DNA encoding the 16 ±4 and 30 ±4 antigens. Thus, the applicants have possession of Sarcocystis neurona DNA encoding the 16 ±4 and 30 ±4 antigens." Appeal Brief, page 7. Appellants argue further that "[c]onstructing and screening an expression library for clones containing DNA encoding a particular protein is routine in the art," and thus "a person of ordinary skill in the art following the applicants' disclosure would have a high expectation of success of recovering clones from an expression library that express the 16 ±4 or 30 ±4

antigens using the antibodies against the 16 ±4 and 30 ±4 antigens prepared as taught in Example 1." Id. at 8.

Appellants' arguments are not convincing. First, the fact that appellants had possession of Sarcocystis neurona is not sufficient to provide possession of DNA that encodes the 16 ±4 and 30 ±4 antigens. As noted above, even a partial amino acid sequence of the 16 ±4 and 30 ±4 antigens, which would necessarily require possession of the source of the DNA, i.e., possession of Sarcocystis neurona, would not be sufficient to provide written description support for the claimed DNA encoding the 16 ±4 and 30 ±4 antigens. In addition, as also discussed above, the existence of a workable method to obtain the DNA sequence is also not sufficient to demonstrate written description support.

With respect to claims 51 and 52, appellants argue that appellants have possession of Sarcocystis neurona DNA, which "would be expected to encode a plurality of antigens, including the 16 ±4 and 30 ±4 antigens. Therefore, when the DNA is inoculated into a horse, the antigens encoded thereon are expressed in the horse." Appeal Brief, page 10. According to appellants, "[c]laims 51 and 52 do not depend on knowing the DNA sequences encoding the plurality of antigens. The claims merely require that the DNA encode one or more Sarcocystis neurona antigens. Thus, the DNA can be the entire Sarcocystis neurona genome(intact or fragmented) or particular DNA fragments therefrom." Id. at 11.

The above argument is also not found to be convincing. The disclosure as filed does not provide written description support for the use of the entire Sarcocystis neurona genome (intact or fragmented) or particular DNA fragments therefrom as a DNA vaccine. The written description is limited to a "DNA vaccine that contains or expresses at least one epitope of an antigen that has an amino acid sequence substantially similar to a unique 16 (±4kDa) antigen and/or 30 (±4) kDa antigen of Sarcocystis neurona." Specification, page 1 (Field of the Invention); see also pages 5, 17, 24 and 26. Thus, the rejection of claims 51 and 52 under 35 U.S.C. § 112, first paragraph, for lack of adequate written description, is affirmed for the reasons set forth supra with respect to the discussion of claims 10-12, 18-20, 44, 45 and 47-50.

2. Rejection under 35 U.S.C. § 112, second paragraph

Claims 51 and 52 stand rejected under 35 U.S.C. § 112, second paragraph, "as being vague and indefinite in the recitation of 'derived'. Is this DNA isolated from S. neurona?" Examiner's Answer, page 9.

This rejection is affirmed in view of appellants' statement that they will amend the term "derived" to "isolated." See Appeal Brief, page 20.

CONCLUSION

The rejection of claims 10-12, 1-20, 44, 45 and 47-52 under 35 U.S.C. § 112, first paragraph, for lack of adequate written description, and the rejection of claims 51 and 52 under 35 U.S.C. § 112, second paragraph are affirmed. Because we affirm the rejection under 35 U.S.C. § 112, first paragraph, on the

basis of lack of adequate written description, we decline to reach the merits of the rejection under 35 U.S.C. § 112, first paragraph, for lack enablement.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 CFR § 1.136(a).

AFFIRMED

William F. Smith)
Administrative Patent Judge)
)
)
) BOARD OF PATENT
Eric Grimes)
Administrative Patent Judge) APPEALS AND
)
) INTERFERENCES
Lora M. Green)
Administrative Patent Judge)

MCLEOD & MOYNE, P.C.
2190 COMMONS PARKWAY
OKEMOS, MI 48864

4.1-526

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte LINDA S. MANSFIELD, MARY G. ROSSANO, ALICE J. MURPHY and RUTH A. VRABLE

Appeal No. 2005-2386
Application No. 09/670,096

ON BRIEF

Before GRIMES, GREEN, and LEBOVITZ Administrative Patent Judges.

GREEN, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 1, 2 and 21,[1] all of the pending claims, which are reproduced below:

[1] An amendment after final was filed concurrently with the Appeal Brief, dated August 20, 2004, and stamped August 23, 2004. Appellants state in the Appeal Brief that an Amendment was filed August 20, 2004, and in response, the examiner in the Examiner's Answer merely states that appellants' statement is correct, but does not explicitely state that the amendment was entered. But because the rejection of claim 2 under 35 U.S.C. § 112, second paragraph, for lack of antecedent basis was withdrawn, and the amendment after final remedied that issue, we infer that the amendment was entered. Thus, the claims as reproduced here are as amended by the August 23, 2004, amendment after final.

c: Mansfield
Hudson
6/6/06

1. A composition for treating an equid infected with Sarcocystis neurona comprising a mixture of isolated antibodies against a 16 ±4 kDa antigen of Sarcocystis neurona and isolated antibodies against a 30 ±4 kDa antigen of Sarcocystis neurona wherein the antibodies are from serum of an animal immunized with the antigen and wherein the mixture is in a pharmaceutically acceptable carrier.

2. The method of claim 21 wherein the antibodies are monoclonal antibodies.

21. A method for treating an equid infected with Sarcocystis neurona comprising:

(a) providing a mixture of antibodies against a 16 ±4 kDa antigen and a 30 ±4 kDa antigen, both of which are specific to Sarcocystis neurona, wherein the antibodies are selected from the group consisting of polyclonal antibodies from serum from an animal immunized with the antigen and monoclonal antibodies from a hybridoma, and wherein the antibodies are in a pharmaceutically acceptable carrier; and

(b) inoculating the equid with the antibodies in the carrier to treat the equid.

The claims stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter that was not described in the specification in such a way so as to enable one skilled in the art to which it pertains or with which it is most nearly connected to make and/or use the invention. After careful review of the record and consideration of the issue before us, we reverse.

DISCUSSION

Claims 1, 2 and 21 stand rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement.

"[A] specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first

paragraph of § 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support." In re Marzocchi, 439 F.2d 220, 223, 169 USPQ 367, 369 (CCPA 1971) (emphasis in original). "[It] is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement." Id. at 224, 169 USPQ at 370. Here, the examiner has not provided "acceptable evidence or reasoning which is inconsistent" with the specification, and therefore has not met the initial burden of showing nonenablement.

In making the enablement rejection, the examiner engages in the analysis of the factors as set forth in In re Wands, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988). Examiner's Answer, page 5.

The examiner notes that given the high rate of exposure of horses to S. neurona and the low incidence of clinical equine protozoal myeloencephalitis (EPM), "indicate[s] that most horses develop effective immunity (no clinical symptoms of disease) that may prevent merozoite entry into the central nervous system." Id. at 6. The examiner goes on to state that the pathogenesis of the disease is not fully understood, and that clinical manifestations of the disease only occur in a small percentage of seropositive horses, citing Cutler[2] in noting

---

[2] Cutler et al. (Cutler), "Immunoconversion against Sarcocystis neurona in normal and dexamethasone-treated horses challenged with S. neurona sporocysts," Veterinary Parasitology, Vol. 95, pp. 197-210 (2001).

that "it is important and necessary to identify factors that govern progression from an apparent infection to clinically evident neurological disease, EPM . . . in horses." Id.

According to the examiner, "[t]he treatment of S. neurona infection in an equid with antibodies is highly complex and unpredictable because relative to the infection, the development of clinical spreading of the disease i.e., merozoite entry into the central nervous system crossing blood brain barrier is not known as most of the horses develop immunity without EPM." Examiner's Answer, page 6. As "the prior art does not teach administration of a mixture of isolated antibodies against a 16 kD antigen of S. neurona and isolated antibodies against a 30 kD antigen of S. neurona to an infected horse with EPM which would resolve the infection in CNS[,] . . . [t]hus there is a lack of understanding in the art with respect to the pathogenesis of S. neurona infection in horses that develop EPM." Id. at 7.

The examiner also relies on Liang 1998[3] to support the proposition that "not all antibodies generated during infection will neutralize the merozites." Examiner's Answer, page 7. The examiner asserts that from Liang 1998 it appears that extended exposure to antiserum appears to be necessary, and that in vitro data do not necessarily correlate to the results that will be obtained in vivo. See id. Moreover, according to the examiner, "it is unclear whether such

---

[3] Liang et al. (Liang 1998), "Evidence that Surface Proteins Sn14 and Sn16 of *Sarcocystis neurona* Merozoites Are Involved in Infection and Immunity," Infection and Immunity, Vol. 66, No. 5, pp. 1834-1838 (1998).

an immunotherapy can be used to treat all horses that are infected with S. neurona." Id. at 8. The examiner is also concerned that the specific antibodies used in the claimed immunotherapeutic methods are not characterized. See id.

We do not find that the examiner has provided evidence and/or reasoning that the claims are not enabled by the specification. As noted by appellants, "since many horses exposed to Sarcocystis neurona do not have clinical signs of EPM but have immunity to Sarcocystis neurona the serum antibodies are likely effective for protecting against the parasite." Appeal Brief, page 9. Given that, as further noted by appellants, "it would appear to be reasonable to believe that horses with EPM have an inadequate immune response to the parasite which is not sufficient to prevent entry of the parasite into the CNS and that boosting the immune response with antibodies against the 16 and 30 kDa antigens might provide a sufficient boost to an infected horse's immune response to inhibit entry of the parasite into the CSF." Id. at 16.

Moreover, Liang 1998 teaches that Sarcocystis neurona is sensitive to specific antibodies, and thus does not support the examiner's contention that the claims are not enabled. In regard to the examiner's statement that "it is unclear whether such an immunotherapy can be used to treat all horses that are infected with S. neurona," there is no requirement that the claimed method work with all horses that are infected with S. neurona.

In addition, Appellants submitted a declaration on April 1, 2003, Appendix B to the Appeal Brief, demonstrating that both the 16 and 30 kDa antigens

appeared to be more neutralizing than either antibody alone. See Appeal Brief, page 14. In response, the examiner argues that although "[t]he Declaration provides evidence that CSF from infected horses contains antibodies to 16kD and 30kD [antigens] and such antibodies neutralize the merozoites in vitro (neutralization assays) only," the declaration "does not provide any evidence that the claimed composition comprising said antibodies are useful for treating an equid infected with S. neurona." Examiner's Answer, page 13.

Thus, the examiner's principal concern appears to be that the specification provides no in vivo examples of treating a horse. See, e.g., Examiner's Answer, pages 6 and 8. The examiner notes that the specification "only discloses that multiple isolates of merozoites have been obtained by culturing sporozoites from opossum," id. at 8, and that "[t]he specification . . . provides no working examples demonstrating . . . enablement for the claimed composition or a method that is required in this under developed art. The specification only teaches culturing sporocysts and merozoites," id. at 9.

The presence or absence of a working example, however, is not determinative on the issue of enablement. It is just one factor that is to be weighed with the other factors. In the case at issue, the examiner has not met the burden of demonstrating that the specification does not enable the claims, and the rejection under 35 U.S.C. § 112, first paragraph, for lack of enablement, is reversed.

OTHER ISSUES

Appellants' and the examiner's attention is directed to related Appeal Number 2004-1976, U.S.S.N. 09/669,843. That appeal contained a claim to:

> A monoclonal mixture comprising an antibody that selectively binds to a 16 ± 4 kDa antigen of Sarcocystis neurona and a monoclonal antibody that selectively binds to a 30 ± 4 kDa antigen of Sarcocystis neurona wherein the antigens are separately isolated from Sarcocystis neurona merozoites by two-dimensional polyacrylamide gel electrophoresis and separately used to produce hybridomas which produce the monoclonal antibodies for the mixture.

In the 2004-1976 appeal, we affirmed a rejection under 35 U.S.C. § 103(a) over the combination of Liang 1998 or Liang 1997[4] and Harlow.[5]

Similarly, in the instant appeal, claim 1 is directed to:

A composition for treating an equid infected with Sarcocystis neurona comprising a mixture of isolated antibodies against a 16 ±4 kDa antigen of Sarcocystis neurona and isolated antibodies against a 30 ±4 kDa antigen of Sarcocystis neurona wherein the antibodies are from serum of an animal immunized with the antigen and wherein the mixture is in a pharmaceutically acceptable carrier.

The recitation of "for treating an equid infected with Sarcocystis neurona" is a statement of intended use, and not a patentable limitation. See In re

---

[4] Liang et al. (Liang 1997), "Micropreparative High Resolution Purification of Proteins by a Combination of Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis, Isoelectric Focusing, and Membrane Blotting," Anal. Biochem., Vol. 250, pp. 61-65 (1997).

[5] Harlow et al. (Harlow, Antibodies, A laboratory Manual, Chapter 6, Col Spring Harbor Press (1988).

Schreiber, 128 F.3d 1473, 1477, 44 USPQ2d 1429, 1431 (Fed. Cir. 1997). Upon return of the appeal, the examiner may wish to consider the patentability of instant claim 1 in view of the references and the rejection as set forth in Appeal Number 2004-1976.

CONCLUSION

Because the examiner has not set forth a prima facie case of unpatentability, the rejection of claims 1, 2 and 21 under 35 U.S.C. § 112, first paragraph, for lack of enablement, is reversed. Upon receipt of the case, however, the examiner may wish to consider the patentability of claim 1 in view of the rejection under 35 U.S.C. § 103(a) as set forth in related Appeal 2004-1976.

REVERSED

Eric Grimes)
Administrative Patent Judge)
)
) BOARD OF PATENT
)
Lora M. Green) APPEALS AND
Administrative Patent Judge)
) INTERFERENCES
)
)
Richard M. Lebovitz)
Administrative Patent Judge)

MCLEOD & MOYNE, P.C.
2190 COMMONS PARKWAY
OKEMOS MI 48864

41-458
529

The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte LINDA S. MANSFIELD, MARY G. ROSSANO, ALICE J. MURPHY and RUTH A. VRABLE

Appeal No. 2004-1976
Application No. 09/669,843

ON BRIEF

Before WILLIAM F. SMITH, GRIMES, and GREEN, Administrative Patent Judges.

GREEN, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 36, 51 and 52. The claims read as follows:

36. A monoclonal antibody that selectively binds to a 16 ± 4 kDa antigen of Sarcocystis neurona wherein the antigen is isolated from Sarcocystis neurona merozoites by two-dimensional polyacrylamide gel electrophoresis and used to produce a hybridoma which produces the monoclonal antibody.

51. A monoclonal antibody that selectively binds to a 30 ± 4 kDa antigen of Sarcocystis neurona wherein the antigen is isolated from Sarcocystis neurona merozoites by two-dimensional

Docketed
Sept 29, 2005

cc: B. Sherman 9/3/06

polyacrylamide gel electrophoresis and used to produce a hybridoma which produces the monoclonal antibody.

52. A monoclonal mixture comprising an antibody that selectively binds to a 16 ± 4 kDa antigen of Sarcocystis neurona and a monoclonal antibody that selectively binds to a 30 ± 4 kDa antigen of Sarcocystis neurona wherein the antigens are separately isolated from Sarcocystis neurona merozoites by two-dimensional polyacrylamide gel electrophoresis and separately used to produce hybridomas which produce the monoclonal antibodies for the mixture.

The examiner relies upon the following references:

Liang et al. (Liang 1997), "Micropreparative High Resolution Purification of Proteins by a Combination of Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis, Isoelectric Focusing, and Membrane Blotting," Anal. Biochem., Vol. 250, pp. 61-65 (1997).

Liang et al. (Liang 1998), "Evidence that Surface Proteins Sn14 and Sn16 of *Sarcocystis neurona* Merozoites Are Involved in Infection and Immunity," Infection and Immunity, Vol. 66, No. 5, pp. 1834-1838 (1998)

Harlow et al. (Harlow), Antibodies, A Laboratory Manual, Chapter 6, Cold Spring Harbor Press (1988).

Claims 36, 51 and 52 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over the combination of Liang 1998 or Liang 1997 and Harlow. After careful review of the record and consideration of the issue before us, we affirm.

DISCUSSION

The claims stand rejected under 35 U.S.C. § 103(a) as being obvious over the combination of Liang 1998 or Liang 1997 and Harlow.

Liang 1998 is cited for teaching the identification of S. neurona merozoite antigens from samples from horses with neurological signs typical of equine

myeloencephalitis (EPM) or confirmed EPM. See Examiner's Answer, page 3. The antigens so identified include a 30 KD antigen and a 16 KD antigen, which, according to the reference, appear to be cell surface antigens of merozoites. See id. The examiner states that "[a] combination of the results of western-blot analysis (figure 1) and trypsin digestion (figure 3 B) suggests that these are important surface proteins that could be used in specific diagnosis of S. neurona infection, as candidate antigens for vaccine development and specific antibodies to these antigens lyse merozoites via complement or inhibit their attachment and penetration to host cells." Id. Liang 1998 is also cited for teaching that monoclonal antibodies are often used to study parasitic proteins. See id. The examiner acknowledges that while "[a]ntibodies to 16KD antigen not only recognized the 16KD antigen but also lysed the merozoites in in vitro neutralization assays . . . antibodies to 30KD recognized the 30KD antigen but could not inhibit in vitro neutralization of merozoites, as 30KD antigen appears to cross-react with serum obtained from horses infected with other Sarcocystis species." Id. at 4. The examiner concludes "[t]hus the prior art teaches 30KD, 16KD, 14KD and 11KD proteins as merozoite surface antigens that are involved in S. neurona infection and EPM and could be used in the specific diagnosis of S. neurona." Id.

Liang 1997 is cited for teaching "purified 30 KD and 19 KD (i.e. 16 KD +/- 4) antigens from S. neurona merozoites by using infected horses serum." Id.

The rejection acknowledges that "Liang 1998 or Liang 1997 does not teach monoclonal antibody that selectively binds to 16KD antigen or a monoclonal antibody that selectively binds to a 30KD antigen or a monoclonal antibody mixture." Id.

Harlow is cited for teaching methods for "making monoclonal antibodies to any given antigen." Id.

The rejection concludes:

> It would have been prima facie obvious to one, having ordinary skill in the art at the time the invention was made to make monoclonal antibodies to merozoite surface antigens including 16KD and 30KD because Liang [ ] taught detection of S. Neurona infection using serum and sometimes infected horse serum cross reacts with antigens such as 30KD. Further, the art suggests monoclonal antibodies are often used to study parasite (page 1837, last paragraph) proteins and EPM disease occurs after merozoite passes through the vascular endothelium of blood-brain barrier into the central nervous system, and so humoral responses play essential role in blocking this migration and specific cytotoxic T cells are ineffective in attacking merozoite migration to the central nervous system in the blood stream (pag[e] [sic] 1837, left column, third paragraph). Therefore an artisan of ordinary skill would have been motivated to use readily available and purified surface antigens (Liang [ ] 1997) from merozoites including 16KD and 30 KD as disclosed by the prior art Liang [ ] 1998 or Liang [ ] 1997 with a reasonable expectation of success for raising monoclonal antibodies by using well established hybridoma technology as taught by [Harlow] because Liang [ ] 1998 suggests that humoral immunity to S. neurona infection is important (see page 1836 under discussion) especially in EPM disease and surface antigens including 30KD, 16KD, 14KD are immunoreactive with infected serum that are useful for the detection of the pathogenic S. neurona and Liang [ ] 1997 teach purification of target merozoite proteins 19 KD, 30 KD and 100KD. Moreover, it has become routine in the art to make monoclonal antibodies for characterizing and purifying proteins especially target proteins such as surface proteins of parasites or envelope proteins of bacteria.

Id. at 4-5.

We initially note that we find the Liang 1997 reference to be cumulative to the Liang 1998 reference, thus we focus our analysis on the Liang 1998 reference.

It is axiomatic that "the Examiner bears the burden of establishing a prima facie case of obviousness based upon the prior art. '[The Examiner] can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references.'" In re Fritch, 972 F.2d 1260, 1265, 23 USPQ2d 1780, 1783 (Fed. Cir. 1992) (citation omitted). An adequate showing of motivation to combine requires "evidence that 'a skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select the elements from the cited prior art references for combination in the manner claimed.'" Ecolochem, Inc. v. Southern Calif. Edison Co., 227 F.3d 1361, 1375, 56 USPQ2d 1065, 1076 (Fed. Cir. 2000). We find that the rejection establishes a prima facie case of obviousness that has not been rebutted by appellants, and the rejection is affirmed.

Appellants argue that the combination does not provide any motivation to make the claimed monoclonal antibodies or the claimed mixture of antibodies. See Appeal Brief, page 6. Appellants acknowledge that "[g]enerating antibodies to a given antigen or epitope may have become routine in recent years," id. at 7,

but contend that "[t]here must also be a clear objective or motivation for one skilled in the art to combine the prior art." See id. Appellants assert that "the prior art references do not identify any need for making antibodies against the [16 and 30] kDa antigens or identify any problem that the antibodies could be used to solve." Id. at 14. Appellants contend that the rejection apparently relied on the "notion that making antibodies is routine" to provide the motivation to combine Liang 1998 with Harlow, which, according to appellants, is an impermissible hindsight rejection. See id. at 14.

Appellants argue with respect to claims 51 and 52 that the ordinary artisan would not have had motivation to make antigens against the 30 kDa antigen, because, while Liang 1998 suggests that the 16 kDa antigen appears to be an important antigen, Liang 1998 "suggests that the 30 kDa antigen is not important because it had no inhibitory activity and antibodies against the antigen were not recognized to be specific." Appeal Brief, page 15 (references omitted). Thus, appellants conclude, the ordinary artisan would not have been motivated to produce monoclonal antibodies against the 30 kDa antigen as in claim 51, or to produce a mixture containing that antibody as in claim 52.

Appellants' arguments are not convincing. As noted by the rejection, Liang 1998 specifically teaches that "monoclonal antibodies are often used to study parasitic proteins." See Liang 1998, page 1837, Col. 2. Moreover, Liang 1998 also teaches that *Sarcocystis neurona* is the etiologic agent of equine protozoal myeloencephilitis, see Liang 1998, page 1834, Abstract and Col. 1,

thus providing motivation to study and detect the parasite. Liang 1998 also teaches that antibodies specific for the 16 kDa antigen have protective activity against *S. neurona* and "support the use of the immunoblot test in diagnosis of [equine protozoal myeloencephilitis]," thus providing motivation to generate antibodies against the 16 kDa antigen.

With respect to the 30 kDa antigen, although Liang 1998 teaches that the antibodies are immunoreactive with sera from horses, infected with other *Sarcocystis*, it also teaches that monoclonal antibodies are often used to study parasitic proteins. Therefore, one of ordinary skill would have been motivated to produce antibodies to the 30 kDa antigen in order to study the antigen in *S. neurona* and other *Sarcocystis*. And one of ordinary skill would understand that a mixture of monoclonal antibodies to the 16 and 30 kDa antigens would allow one to determine if *Sarcocystis* other than *S. neurona* were present in horses with equine protozoal myeloencephilitis.

Appellants argue further that the combination of Liang 1998 and Harlow does not enable one skilled in the art to prepare antibodies against the 16 and 30 kDa antigens. See Appeal Brief. While, according to appellants, Liang 1998 demonstrates that the antigens are antigenic in horses, Harlow relates to the production of monoclonal antibodies in mice, and there is no teaching or suggestion in Liang 1998 that the antigens would produce antibodies, i.e., be antigenic, in mice. See id. at 11-12. Appellants distinguish Ex parte Erlich, 3 USPQ2d 1001 (Bd. Pat. App. & Int. 1986) by arguing that "because even though

Liang . . . (1998) show[s] that the 16 and 30 kDa antigens are antigenic, there is no prior art which shows that the method of [Harlow] could be adapted to produce monoclonal antibodies against the [16 and 30] kDa antigens." Id. at 13-14. Appellants conclude that the prior art does not provide a reasonable expectation of success that monoclonal antibodies could be generated against the 16 and 30 kDa antigens. See id. at 14.

Again, appellants' arguments are not found to be convincing. As acknowledged by appellants, methods for obtaining and screening for monoclonal antibodies were well known at the time of invention. See also In re Wands, 858 F.2d 731, 736, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). Because the 16 and 30 kDa antigens from *S. neurona* were antigenic in horses, one of ordinary skill in the art would expect them to be antigenic in other mammals, such as mice. Moreover, appellants themselves refer to the 16 and 30 kDa proteins from *S. neurona* as antigens, and define an antigen as "a substance which stimulates production of antibody or sensitized cells during an immune response," Specification, page 12, thus one of ordinary skill would expect the 16 and 30 kDa antigens from *S. neurona* to be antigenic in mice. Finally, all that is required is a reasonable expectation of success, not absolute predictability of success. See In re O'Farrell, 853 F.2d 894, 903, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988).

CONCLUSION

Because the examiner has established a prima facie case of obviousness that has not been rebutted by appellants, the rejection of claims 36, 51 and 52 under 35 U.S.C. § 103(a) is affirmed.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 CFR § 1.136(a).

AFFIRMED

William F. Smith
Administrative Patent Judge)
)
)
)
Eric Grimes) BOARD OF PATENT
Administrative Patent Judge) APPEALS AND
)
) INTERFERENCES
)
Lora M. Green)
Administrative Patent Judge)

MCLEOD & MOYNE, P.C.
2190 COMMONS PARKWAY
OKEMOS, MI 48864